JTCM 中医杂志

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J Tradit Chin Med 2014 December 15; 34(6): 641-645
ISSN 0255-2922

CLINICAL STUDY

Effect of ginseng polysaccharides and dendritic cells on the balance of Th1/Th2 T helper cells in patients with non-small cell lung cancer

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Supported by 2011 National Key Specialty Construction of Clinical Projects of China; The National Natural Science Foundation of China (No. 81273639); Science and Technology Projects of Traditional Chinese Medicine Bureau in Jiangsu Province of China (No. LB13042)
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Accepted: January 22, 2014

Abstract

OBJECTIVE: To investigate the effect of thoracoscopic administration of ginseng polysaccharides (GPS) plus dendritic cells (DC) on T helper cell type 1/T helper cell type 2 (Th1/Th2) balance in patients with non-small cell lung cancer (NSCLC).

METHODS: A total of 96 NSCLC patients were divided evenly into two groups. The control group was treated with DCs alone and the treatment group was treated with DCs plus GPS. After DCs and GPS were administered thoracoscopically, once a week, 4 times for 30 days, the patients' quality of life was measured with the Functional Assessment of Cancer Treatment-Lung (FACT-L) questionnaire before and after treatment. Serum interferon-γ (INF-γ), interleukin-4 (IL-4), IL-2 and IL-5 were examined before and after treatments.

RESULTS: The level of Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4, IL-2/IL-5) increased in both treatment groups, while Th2 cytokines (IL-4, IL-5) and FACT-L scores decreased (P<0.01). Furthermore, after treatment Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4, IL-2/IL-5) were higher in the DCs + GPS group than in the control group (P<0.05). Conversely, FACT-L scores and Th2 cytokines (IL-4, IL-5) were higher in the control group than in the DCs + GPS group (P<0.05).

CONCLUSION: The treatment regime of DCs plus GPS had a greater effect on NSCLC patients' immune function as compared with DCs alone. This was evident by increased expression of Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 (INF-γ/IL-4, IL-2/IL-5), as well as by decreased FACT-L scores and the expression of Th2 cytokines (IL-4, IL-5).

Key words: Panax; Dendritic cells; Carcinoma, non-small-cell lung; Interferons; Interleukins

INTRODUCTION

Non-small cell lung carcinoma (NSCLC) is one of the most malignant tumors and has the highest mortality rate in China. In addition to traditional treatments including surgery, radiotherapy, chemotherapy and mo-

lecular targeted therapy, recently immunotherapy has gradually been gaining attention as a treatment option.[1] Dendritic cells (DCs), widely known for their role as antigen presenting cells (APCs),[2] also have anti-tumor functions achieved by regulating immune function. Previous studies have focused on the subcutaneous, venous and lymphatic administration of DCs.[3] However, thoracoscopy was shown to be the optimal method for administration.[4]

Ginseng polysaccharides (GPS) have been shown to regulate the immune function of DCs in NSCLC patients.[5] However, no studies on the effect of GPS plus DCs administered thoracoscopically have been performed. Therefore, the present study was conducted in NSCLC patients examining the Functional Assessment of Cancer Treatment-Lung (FACT-L) scores, Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4,IL-2/IL-5)[6,7] in BenQ hospital of Nanjing.

MATERIALS AND METHODS

Patients

A total of 96 patients diagnosed with NSCLC were recruited between January 2010 and July 2013 from BenQ hospital and randomly divided into two groups according to random number table: the control group and treatment group, with 48 patients per group. All patients were informed and the written informed consent forms were obtained. The study was approved by the ethics committee of BenQ hospital of Nanjing. The baseline characteristics between the two groups were not statistically significant (P>0.05, Table 1).

Diagnostic criteria

Diagnosis of NSCLC was performed following the World Health Organization lung and pleura tumor histologic classification criteria revised in 2004[8] and according to the International Union of Cancer (UICC) and the American Association of Cancer (AJCC) diagnostic criteria.[9]

Inclusion criteria

The inclusion criteria for the study were (a) meeting the diagnosis criteria for NSCLC, according to tumor staging international standards (TNM) for stage ⅢA and ⅢB, pathological classification for adenocarcinoma, nodular type; (b) having undergone first surgery and three to six cycles of chemotherapy regimens for NP, GP or EP; (c) cessation of chemotherapy for between 1 and 2 months prior to commencement of the study; (d) aged between 30 and 70 years old; (e) Karnofsky Performance Status (KPS) scores greater than 60 points; (f) signing informed consent form.

Exclusion criteria

The exclusion criteria for the study were (a) postoperative distant metastasis; (b) severe conditions of heart, cerebrovascular, liver, kidney or hematopoietic systems; (c) congenital disease or mental illness; (d) pregnancy or lactation.

Treatment

All cases were taken the foundation treatment such as postoperative symptomatic, supportive and targeted (erlotinib hydrochloride tablet, 150 mg/d, po.) remedy, there was no difference between the foundation treatment schemes adopted in each group (P>0.05). On the basis of the treatment, the control group was treated with DCs alone and the treatment group with GPS plus DC under the thoracoscope.

Induction and self antigen load of DCs in peripheral blood: according to the reference,[10] collect the whole

Table 1 Baseline clinical characteristics between patient groups (n=48, $\bar{x} \pm s$)

Baseline		Control group	Treatment group	P value
Age (years)	Range	32-65	33-66	>0.05
	Mean±standard deviation	51.4±12.3	54.5±13.2	-
Sex (n)	Male	32	31	>0.05
	Female	16	17	-
TNM stages	ⅢA	36	34	>0.05
	ⅢB	12	14	-
Chemotherapy times prior to research		4.1±1.4	3.9±1.4	>0.05
Once chemotherapy regimens	NP	40	39	>0.05
	GP	6	6	-
	EP	2	3	-
Months after chemotherapy was stopped prior to research		1.2±0.3	1.3±0.3	>0.05

Notes: control group was treated with DCs only; teatment group was treated with GPS plus DC. NP: vinorelbine and cisplatin; GP: gemcitabine and cisplatin; EP: etoposide and cisplatin; TNM: tumor staging international standards; GPS: ginseng polysaccharides; DC: dendritic cells.

blood of patients with anticoagulant 100 ml, and collect 2 h-post wall composition of PBMNC (Peripheral Blood Mononuclear Cell) as precursor cells, With 2 × 10^6/mL concentration to join serum-free medium AIM-V with gm-csf 1000 U/mL, IL-4 1000 U/mL. To cultivate at the environment of 37℃, 5% CO_2, replace half quantity of culture liquid every 3 day. Cultured cells 5 days, add stimulus with tumor antigen 0.5 mL, At the same time to collect the patient's own tumor tissue in 5 days, join the TNF-α (tumor nectosis factor-alphagene) 20 ng/mL in the seventh day, DCs with tumor antigen was acquired in the eighth day. On this basis, To prepare for the next stage of treatment, the number, survival rate, sterile, gram staining, endotoxin, mycoplasma, purity and uniformity of cell quality of DCs were detected.

Injection of DC or GPS add DC under the thoracoscope: the Storz type 260 thoracoscope (Karl Storz GmbH & Co., Tuttlingen, Germany) and the Stryker imaging system (Stryker, Kalamazoo, MI, USA) were used for thoracoscopic administration of DCs and GPS. DC cells number is 0.06×10^6/kg, GPS is 0.5 mg/kg, once a week, 4 times for 30 days. GPS injection was bought from Shanxi Pude pharmaceutical Co., Ltd., Shanxi, China; DCs was obtained from labolatory of Jiangsu shuyang hospital of TCM, Jiangsu, china. Indwelling 1 tube in chest drainage after the first injection, and arranged another injection with thoracoscope, strict control of infection, and strengthen the nursing of chest indwelling catheter for the prevention and treatment of pleural adhesion in the course of treatment,and given the extubation after treatment.

Data collection

Before and after treatment, the FACT-L scores were measured at dawn and 3 ml of venous blood collected in the morning, centrifuged force at 2220 ×*g* for 10 min, and stored at - 75℃. After clotting, serum INF-γ, IL-4, IL-2, IL-5 levels were determined with double sandwich ELISA (enzyme-linked immuno sorbent assay) kits according to the manufacturer's instructions (Beijing Sizhengbai Biological Technology Co., Ltd., Beijing, China). ELISA plates were read on a Rainbow type spectrophotometer (Tecan Group Ltd., Mannedorf, Switzerland).

Statistical methods

Data are presented as the mean ± standard deviation (*SD*). The means of the two groups were compared using a *t* test. Statistical analysis was performed using SPSS version 19.0 (IBM Co., Ltd., Armonk, New York, USA).

RESULTS

FACT-L scores before and after treatment

The mean FACT-L score of each group decreased significantly after treatment (P<0.01). Furthermore, after treatment the mean FACT-L score in the control group was higher than that in the DC+GPS group (P< 0.05, Table 2).

Expression of Th1 and Th2 cytokines in sera before and after treatment

The expression of Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4,IL-2/IL-5) increased in each group with treatment, while Th2 cytokines (IL-4, IL-5) decreased significantly (P< 0.01). Additionally, after treatment Th1 cytokines (INF-γ, IL-2) and the ratio of Th1/Th2 cytokines (INF-γ/IL-4, IL-2/IL-5) were greater in the DC+GPS treatment group than in the control group, while conversely Th2 cytokines (IL-4, IL-5) were higher in the control group than in the DCs + GPS treatment group (P<0.05, Table 3).

DISCUSSION

Our study revealed that the FACT-L score in patients decreased after both interventions, and that the effect of GPS plus DCs was greater than that of DCs alone. This suggests that GPS interact with DCs to stimulate the immune system and that the secretion of cytokines is involved in the regulation of the immune response to NSCLC. Th1 and Th2 are the two major subtypes of CD4+ T lymphocytes. Th1 is linked to cell-mediated immunity and Th1 cells produce INF-γ and IL-2 and inhibit tumor cell proliferation. Th2 mediates the humoral immune can produce IL-4 , IL-5, and the other cytokines. In contrast to the Th1 response, the Th2 response can actually promote tumor cell prolifera-

Table 2 FACT-L scores in the two patient groups before and after treatment (scores, n=48, $\bar{x}\pm s$)

Group		Physical status	Community/ family status	Emotional status	Functional status	Additional status	Total
Control	Before	22±4	25±7	17±5	20±5	16±5	99±22
	After	17±4[a]	18±5[a]	13±4[a]	15±4[a]	12±4[a]	77±15[a]
Treatment	Before	23±5	24±6	19±6	19±6	18±6	98±22
	After	14±3[ab]	1 5±3[ab]	11±3[ab]	13±3[ab]	10±3[ab]	65±16[ab]

Notes: control group was treated with DCs alone; the treatment group was treated with GPS plus DCs. GPS: ginseng polysaccharides; DC: dendritic cells; FACT-L: functional assessment of cancer treatment-lung. Compared with the indexes before treatment in each group, [a]P<0.01; compared with the indexes after treatment in control group, [b]P<0.05.

Table 3 Expression of Th1 and Th2 cytokines in the two patient groups before and after treatment (ng/L, n=48, $\bar{x} \pm s$)

Group		INF-γ	IL-4	INF-γ/IL-4	IL-2	IL-5	IL-2/IL-5
Control	Before	34.13±9.24	49.54±9.26	0.69±0.22	25.15±6.14	62.58±12.14	0.40±0.15
	After	65.36±10.21[a]	39.34±9.12[a]	1.66±0.72[a]	51.56±9.48[a]	53.25±12.37[a]	0.98±0.38[a]
Treatment	Before	32.46±9.65	47.88±9.67	0.68±0.17	24.12±6.98	61.34±14.29	0.39±0.17
	After	75.35±12.86[ab]	32.54±8.32[ab]	2.32±0.99[ab]	62.42±17.97[ab]	43.37±11.32[ab]	1.44±0.62[ab]

Notes: control group was treated with DCs alone; treatment group was treated with GPS plus DCs. INF-γ: interferon-γ; IL-4, IL-2, IL-5: interleukin-4, -2, -5; GPS: ginseng polysaccharides; DC: dendritic cells; ELISA: enzyme-linked immuno sorbent assay. Cytokine concentration (ng/L) was determined by ELISA and is presented as the mean±standard deviation. Comparison with the indexes before treatment in each group, [a]P<0.01; comparison with the the indexes after treatment in control group, [b]P<0.05.

tion.[11] Th1/Th2 cells are in a state of relative balance under normal circumstances. However, owing to abnormal immune function in patients with tumors, the dynamic balance of Th1/Th2 cytokines is altered, mainly with decreased function of Th1 and increased function of Th2 cells.[12]

The DC has been the focus point of the field of biological tumor treatment in recent years.[13,14] DC treatment aims to regulate the body's immune function by stimulating and enhancing specific anti-tumor immune response in patients with various forms of cancer.[15] Previous studies revealed that DCs regulated immune function in NSCLC patients through the balance of Th1/Th2 cytokines.[16] Ginseng polysaccharides are antitumor agents isolated from Renshen (*Radix Ginseng*) that act through immune regulation.[17] Our findings demonstrate that Th1 and Th2 cytokines and the ratio of Th1/Th2 in the two groups were significantly changed after treatment. Specifically Th1 cytokines and the ratio of Th1/Th2 cytokines increased, while Th2 cytokines decreased. The effect evident in the GPS plus DCs group was greater than that of DCs alone. This may be evidence of synergism between GPS and DCs in regulating the immune function.

Based on the above findings, the positive effect of GPS plus DCs on NSCLC patients' immune function was greater than that of using DC alone. This suggests that there is synergy between GPS and DCs when they were administered together.

ACKNOWLEDGEMENTS

We Appreciate the Jiangsu Shuyang Hospital of Traditional Chinese Medicine which provided the technology of induction and self antigen load of DCs.

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